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The Folding of an Immobile DNA-Branched Junction

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Beamline(s): X28C

The central molecular paradigm in genetic recombination is the Holliday junction. This is a structure in which four DNA strands pair to form four double helical arms that flank a branch point. In the presence of Mg^{2+}, the junction folds into a structure where the four arms stack on each other pairwise to form two double helical domains. By contrast, in the absence of Mg^{2+}, or when bound to RuvA, a fourfold symmetric DNA structure is seen. The hydroxyl radical cleavage pattern is sensitive to the difference between these two structures. Relative to their pattern in linear duplex DNA, the two non-crossover strands are protected from attack at sites four nucleotides 3' to the junction in the two-domain structure, because these sites are occluded by the other domain. In the absence of Mg^{2+}, these sites are no longer protected; hence, the folding of this key intermediate is measurable through these differences. The work of a previous student in our laboratory revealed that the stacking reaction rate is about 1.6 sec^{-1}, at room temperature. Since this work was performed on X9, several preliminary control experiments have been repeated on X28C with the new experimental set-up. In addition to reproducing the experiments to determine the stacking rate, future plans also include measuring the influences of temperature and arm-length on the folding rates. In addition, the unstacking rate will be examined by using EDTA to remove Mg^{2+}.